NOTES ON

PRACTICAL AND CLINICAL BIOCHEMISTRY

FOR:

MEDICAL STUDENTS

By:

Dr. FAROUK HUSSEIN SABER

Professor and head OF

DEPARTMENT OF MEDICAL BIOCHEMISTRY

AL-AZHAR UNIVERSITY

2006 - 2007

Second Edition

DEDICATED TO :

* THE SOUL OF MY PARENTS .
* ALL MY FAMILY MEMBERS FOR THEIR PATIENCE AND ENCOURAGEMENT UNTILL THIS BOOK WAS BORN .

PREFACE

- These notes is specially written to meet the needs of medical students. Many updating knowledge in practical & clinical biochemistry were tried.
- Leading questions and their answers were included in this notes as a guide in the practical work.
- Finally, thanks to all members of the medical biochemistry, faculty of medicine, Al-Azhar University, who sincerely supported this book.

Farouk Saber
Prof. and Head of
Medical Biochemistry

CONTENTS

I- LABORATORY INSTRUCTIONS

Practical Biochemistry is of outstanding importance. Every student must be keen in doing the laboratory experiments of each practical lesson. Laboratory instructions are to be followed, viz:

1) Read carefully the label on any reagent bottle before use.
2) Take only the minimum amount of the reagent required to carry out the experiment.
3) Do not replace any solution in a reagent bottle.
4) Stopper immediately the reagent bottle after use making sure not to mix the stoppers.
5) Do not remove special reagent bottles from side benches.
6) Do not put chemicals back in reagent bottles.
7) Make sure that test tubes, pipette, burettes or any glass apparatus aree clean in order to obtain reliable results.
8) Remember that burners must be all turned off before using such inflammable solvents as ether, benzene, etc. incase of fire, the flame is suddenly and completely covered with a piece of cloth.
9) Never pipette strong acids or alkalis. Such corrosive fluids must be taken by a measuring cylinder or burette.
10) Do not throw any waste material in the sinks but is placed in the waste boxes.
11) At the end of each practical lesson, turn off the burner and leave your bench clean and dry.
12) Experimental observations must be recorded in a systematic way in the form of brief but complete statements or properly labeled tables according to the nature of the experiment. Recorded observations must be checked by your demonstrator before leaving the laboratory.

II- MEASUREMENTS

What Is meant by:

1) Normal solution =

$$\frac{\text{Mol. Wt X 1000}}{\text{Sp. Gravity X conc of solution}}$$

2) Molar Solution =

Is mol. Wt in grams dissolved in one liter.

3) Milli equivalent / liter =

$$\frac{\text{Mg of substance / Litre X valence}}{\text{Atomic wt of that substance}}$$

4) Milli litre = 1/1000 litre = 10^{-3}L

5) Micro litre = 1/10^6 litre = 10^{-6}L

6) Kilogram = 1000 grams = 10^3gm

7) Milligram = 1/1000 gm = 10^{-3}gm

8) Microgram = 1/10^6 gm = 10^{-6}gm

9) Blank Solution = Is original solution to be fixed at zero in colorimetry

10) Standard Solution = Is known concentration solution to compare it with unknown test solution.

III- BUFFER SOLSUTIONS

Definition :

They are solutions resist changes in their pH, is spite of adding moderate amount of acids or basis, pH remains constant.

Composition :

- Weak acid, and its salt with strong base ($H_2Co_3/NaHCo_3$) (1:20 = pH 7.4) as in blood buffer. Or,
- Weak base and its salt with strong acid (NH_4OH/NH_4CL) such as ammonium hydroxide > ammonium chloride.

Biochemical Importance :

1) Any slight change in blood pH $\longrightarrow$ acidosis or alkalosis.
2) Each enzyme has its optimum pH to act.
3) All bacteria act at its optimum pH.

Experiment :

Two 1/15 M phosphate buffers are prepared :
1) 1/15 M Acid potassium phosphate KH_2PO_4.
2) 1/15 M Alkaline Sodium Phosphate Na_2HPO_4.

Different proportions of above two buffers are prepared to get different pH solutions as follows:

Different Standard Buffer Solutions

Tube NO.	1	2	3	4	5	6	7	8	9	10	11	12	13
Acid Phosphate	10	9.5	9	8	7	6	5	4	3	2	1	05	–
Alkaline phosphate	–	05	1	2	3	4	5	6	7	8	9	9.5	10
pH	4.6	5.6	5.9	6.3	6.5	6.6	6.7	6.9	7.1	7.3	7.7	7.8	9.2

Answer the following :

- What is acid?

  Which gives proton $[H]^+$ in solution, $HCL \longrightarrow CL^- + [H]^+$

- What is base?

  Is compound which give (–OH) ion in solution,

  $[NaOH \longrightarrow Na^+ + \{OH^-\}]$

- Amphoteric substance?

  Is substance which acts as acid & alkali.

  $H_2O \rightleftharpoons [H]^+ + [OH]^-$

- PH :

  Is negative log of hydrogen ion concentration = – log $[H]^+$.

IV- INDICATORS

Definition :

Are weak organic acids or bases, posses one colour in ionized form, and another colour in non ionized form.

Such as phenolpthalein is colourless in acid media, red colour in alkaline media.

Importance :

To identify the pH of any biological solution, and its pH range.

Experiment :

Add one drop of each indicator to one ml of each standard buffer solution in the previous experiment.

Note the colour at different pH and record according to table of indicators.

Indicator	pH range	Acid colour	Alkaline colour
1- Methyl red	4.3 – 6.3	Red	Yellow
2- Bromo cresol purple	5.2 – 6.8	Yellow	Purple
3- Bromothymol blue	6 – 7.6	Yellow	Blue
4- Phenol red	6.8 – 8.4	Yellow	Red
5- Cresol Red	7.2 – 8.8	Yellow	Red
6- Thymol blue	8 – 9.6	Yellow	Blue
7- Phenolphtalein	8.3 – 10	Colourless	Red

Record the results :

V- ADSORPTION

Definition :

Is attraction of small particles (as I_2) on surface of large particles as starch to give a blue complex, also talc powder, charcoal act as adsorbent.

Medical importance :

- Charcoal, and dry starch (raw) can absorb gases and bacterial toxins from colon.

Experiment :

Adsorption of dyes by charcoal :

Add a small amount of activated charcoal to 5 ml of 0.01% methylene blue solution or urine. Shake well and filter.

Note that charcoal adsorbs the dye. Other adsorbents are: talc powder, clay and raw starch.

VI- DETECTION OF ELEMENTS IN AN ORGANIC COMPOUND

1) Detection OF Carbon:

Heat small amount of sugar in dry clean test tube until charring, water is evaporated, and carbon is remained as black ash.

$$\underset{\text{sugar}}{C6\ [H2O]6} \xrightarrow[-H_2O]{\text{Heating}} \text{Carbon as ash.}$$

2) Detection OF Nitrogen:

To 5 ml of clean organic compound (albumin) add 0.5 ml of 5% ferrous sulphate and two drops of dil Na oH boil to one minute, and cool.

Acidify with two drops of diluted Hcl, and add a drop of 5% ferric chloride, blue colour or precipitate of Prussian blue indicates the presence of nitrogen. N & C in organic compound combine with Na to form Na cyanide. Ferrous sulphate with Na oH forms ferrous oH.

Ferrous oH + Na cyanide ⟶ Na ferrocyanide.

Na ferrocyanide + ferric cL + Hcl ⟶ Ferric ferrocyanide (Prusslan blue)

3) Detection OF Sulpher:

To 4 ml of clear organic solution, add 3 drops of 10% Na_2Co_3, add tw drops of fresh 5% sodium nitroprusside. A purple violet colour indicates the presence of sulphur.

4) Detection OF Halogens:

- To 4 ml of clear organic solution, add ½ ml dilute nitric acid, boil for 15 minutes, add 2ml 10% silver nitrate solution.
- A curdy white ppt. indicates chloride, (silver chloride). Or
- Yellow ppt. indicates iodide, (silver iodide). Or
- Yellowish white ppt. indicates bromide (silver bromide).

** If tests for nitrogen and sulpher are negative acidify the organic solution with dilute nitric acid.

PROTEINS

Definition:

Are organic nitrogenous compounds, consist of large numbers of alpha amino acids linked together through peptide bonds.

Composition:

C [about 56%], H [7%], O [22%], N [14-20%] and S [0-4%].

Classification:

1) Simple proteins: by hydrolysis give rise to α-amino acids only.
2) Conjugated proteins: proteins, in addition to CHO., lipids or phosphorous.
3) Derived protein: are derived from proteins by action of acids, alkalies or enzymes as peptone, and gelatin.

Detection of proteins:

By Biuret test (at least two peptide bonds must be present; tripeptides)

1) Colour Reactions

1) Biuret's test:

It is a general test for all proteins except amino acids and dipeptides, why?

To 3 ml of protein solution, add 3 ml of 10% NaoH, mix, add 2 drops of 1% copper sulphate solution. A pink or violet colour indicates presence of protein.

2) Xanthoprotein test:

To 2 ml of protein solution, add 2 ml of cone, nitric acid, boil 5 minutes in water both, a yellow colour indicates presence of aromatic amino acids of protein, e.g., tyrosine, phenylalanine, and tryptophane.

3) Millon's test:

To 2 ml of protein solution, add 2 drops of millon's reagent, a white ppt. is formed which turns to brick red on boiling. The test is positive in proteins containing tyrosine.

4) Rosenheim's test:

To 2 ml of protein solution, add 2 drops of Rosenhein's regent, mix well, carefully pour down 2 ml of conc. Sulphuric acid on side of the test tube. A purple ring indicates the presence of tryptophane in protein solution. Gelatin gives a negative test [deficient in tryptophane].

5) Sulphur test:

To 2 ml of protein solution, add 2 ml of 20% NaoH and boil for 5 minutes. Cool, and acidify with few drops of glacial acetic acid. Then add few drops of 5% lead acetate. A dark colour or ppt. of lead sulphide indicates the presence of sulpher amino acids [Cysteine & Cystine]. Methionine does not give this test (strong sulpher bond to methionine).

II) Coagulation Test

1) Heat coagulation test:

- Fill two thirds of test tube with protein solution, heat gently the upper half of portion solution ⟶ coagulum.
- Addition of few drops of 2% acetic acid helps coagulation. Why?
  To achieve its zwitter ion ⟶ max ppt.
- What are the coagulated proteins? ⟶ (Albumin and globulins).

III) Precipitation reactions

1) Precipitation by complex acids:

a) 25% sulphosalicylic acid: Add few drops of sulphosalicylic acid reagent to protein solution ⟶ opacities.

b) 10% Tricholoroacetic acid.

c) Saturated picric acid.

d) Phosphotungestic acid 10%.

2) Precipitation by heavy metals:

a) 1% lead acetate solution.

b) 1% silver nitrate.

c) 3% ferric chloride.

3) Precipitation by absolute alcohol:

By dehydration and precipitation of some proteins.

4) Precipitation by neutral salts (salting out):

a) Nacl (saturated solution).

b) Ammonium sulphate solution:

I) Full saturation:

Add solid ammonium sulphate to tube containing albumin solution, shake until full saturation ⟶ albumin ppt.

II) Half saturation:

Prepare saturated solution in water, mix equal volumes of that solution and globulin solution ⟶ globulin ppt.

Conjugated proteins:

- Casinogen is rich in phosphoproteins.
- Casinogen is soluble in dilute alkalies, not coagulated with heat, gives negative sulphur test.

Derived proteins:

Peptone:

Precipitated by tannic acid or ethanol, not by ammonium sulphate or heating.

Gelatin:

Soluble in hot water, insoluble in cold water, forms reversible heat gel.

Gel test:

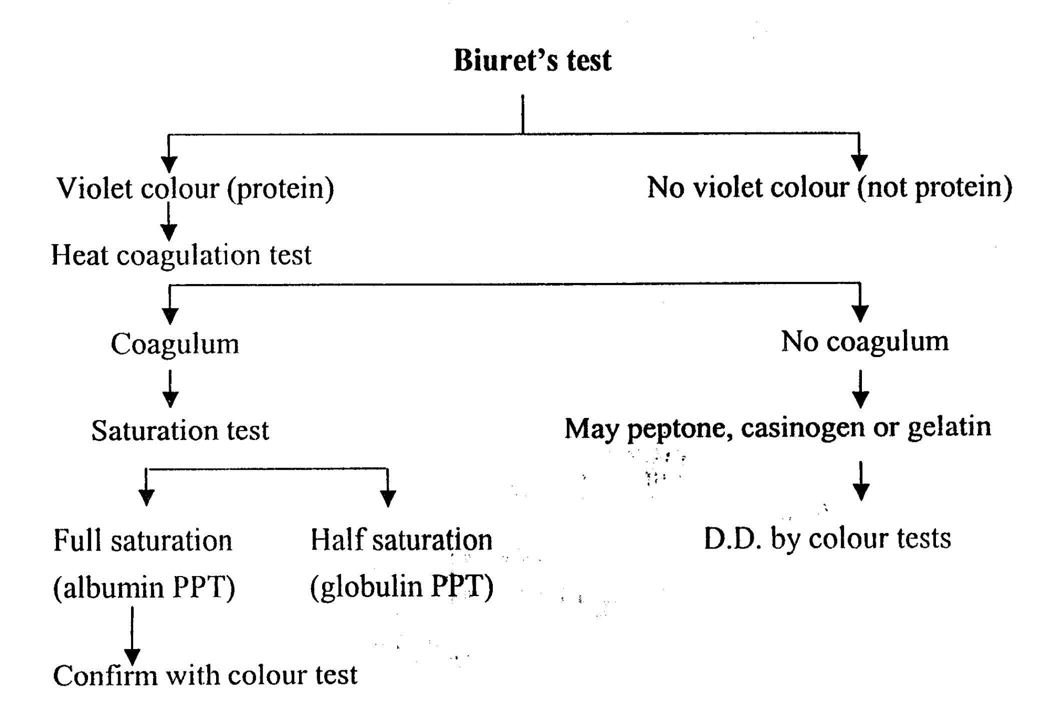
Dissolve gelatin in hot water, and allow it to cool ⟶ gel is formed.

SCHEME FOR IDENTIFICATION OF PROTEINS

I) Physical properties:

1) Colour.
2) Odour.
3) Solubility.
4) PH.

II) Chemical properties:



U R E A

Definition:

Is nitrogenous compound in blood and urine, water soluble, end product of protein catabolism and formed in liver from ammonia.

Experiment:

1) Sodium hypobromite test:

To 5ml of urea solution, add 2ml of alkaline Na hypobromite, mix ⟶ efferescence (nitrogen gases).

$H_2N.Co.NH_2 + 3\ Na\ OBr \longrightarrow 3\ + NaBr + N_2 + Co_2 + 2H_2O$

URIC ACID

Definition:

- Is nitrogenous compound, water insoluble, so may form uric acid stones in urine (acid media).
- Reducing agent [has-C = O group].
- Present in blood (slightly alkaline) as Na urates, so in blood is more soluble.

Source:

1- Purine bases in diet (protein and fish diets, nuclei of plant cells, and xanthines).

2- Wear and tear of human cells.

3- Pathologically in cases of leukemia & cancers (increase cells destruction).

Experiment:

1) Folin's test

To 3ml sodium urate solution, add 1ml of Folin's phosphotungestic acid reagent, and ½ ml of 10% NaoH, a blue colour is formed due to reduction of phosphotungestate by uric acid, forming blue complex tungsten.

CARBOHYDRATES

Definition:

Are polyhydroxyl alcohols containing active aldhyde group (-CHo) or active ketone group (-C=O), are derived form CO_2 & H_2O by photosynthesis.

Structure:

C, H, and O, the latter two H, O are present in the same ratio of water H : O = 2 : 1; so its formula is $C_6 (H_2O)_6 = C_6 H_{12} O_6$.

Classification:

1) Monosaccharides: one sugar unit, as glucose, fructose.
2) Oligosaccharides: 2 – 6 units, as maltose, sucrose and lactose.
3) Polysaccharides: more than six units as starch and glycogen.

Importance:

- Carbohydrates give 60% of all body energy, its excess is stored as liver glycogen.
- Enter in formation of nucleic acids (Ribose).
- Enter in structure of skin & bones (mucopolysaccharides).
- Enter in structure of vitamin B_2 (Ribitol).

Body distribution:

- Glucose (blood)
- Fructose (semen, foetal blood),
- Lactose (breast milk in females).
- Glycogen (liver & muscles).
- Mucopolysaccharides (Connective tissues).
- Glycoproteins (cell receptors and gamma globulins).

MONO & DISACCHARIDES

Experiments:

1) Molisch's Test

Is a general test for all carbohydrates, to 3ml of carbohydrate solution in a clean test tube, add 3 drops of alcoholic alpha-naphthol solution, mix, and add slowly with caution 3ml conc. Sulphuric acid down on the side of test tube ⟶ Reddish violet ring at junction of the two solutions ⟶ carbohydrate.

i) What is the base of this test?

2) Benedict's Test:

Is specific test for all reducing sugars. To 3ml of Benedict's reagent, add 1ml sugar solution, mix, boil for 2 minutes, a green, yellow orange or red ppt. indicates presence of reducing sugars.

ii) What is the chemical nature of red PPT.?

3) Barfoed's Test:

Is more specific test to differentiate between reducing mono & disaccarides.

To 3ml of Barfoed's reagent in test tube, add 1ml of sugar solution, mix, ⟶ boil for 5 minutes in water bath, and cool. Examine against dark background slight reddish PPT. on the side wall of the test tube ⟶ reducing monosacc-harides (within 5 minutes).

iii) Reducing disaccharide give result after boiling than five minutes, Why?

4) Ketose Test:

- Is specific for sugars contain active Ketone group ($-\overset{|}{C}=O$)
- To 3ml of sugar solution add 3ml conc. Hcl, boil 5 minutes in water bath ⟶ orange colour is produced.

iv) Sucrose can give that colour after 5 minutes boiling; Why?

Answers of the practical questions

i) Base of Molish's Test:

Carbohydrates solution + conc. $H_2SO_4 \xrightarrow[-3H_2O]{\text{dehydration}}$ cyclic

Furfural compound + alpha-naphthol $\xrightarrow{\text{condense}}$ violet colour as ring.

ii) Nature of Red PPT in Benedict's Test:

Is red cuprious oxide (CU_2O): $2CU(OH)_2 + R\text{-}CHO \longrightarrow CU_2O + R\text{-}COOH + 2H_2O$

blue capric.OH ⟶ red caprous oxide

iii, iv) The di-saccharides:

Take longer time required for breakdown of di-saccharide ⟶ mono-saccharids, then the mono-S reacts with its specific chemicals.

POLYSACCHARIDES

Definition:

Are collection of monosaccharide units more than six, may be homo-polysaccharide as starch & glycogen, or mixed as glycosaminoglycans & glycoproteins.

Properties:

Non reducing, optically active, give +ve Molish's test.

Importance:

- o Glycogen acts as energy store in liver.
- o Glycosaminoglycans enter in structure of body connective tissues & bones.

Experiments:

1) Iodine test :

- To 5ml of polysaccharide solution, add one or two drops of dilute iodine solution.
- Intense blue colour is produced in case of starch or reddish purple colour if dextrin.

2) Hydrolysis of starch solution by dilute acid :

- To a set of six tubes, add 4ml of 1% starch solution t each tube.
- To each tube add 1ml dilute sulphuric acid except the first tube.
- Immerse in a boiling water bath, and remove tubes at intervals 0,5,10,15, 20 ad 25 minutes.
- Then carry out iodine & Benedict's tests on each tube, record your results with comment?

	Starch	**Amylo-dextrin**	**Erythro-dextrin**	**Achro, Dext**	**Maltose**
Iodine test	Blue	Purple	Red	Colourless	Colourless
Benedict's test	—	—	—	—	Red ppt.

SCHEME FOR IDENTIFICATION OF CARBOHYDRATES

i] Physical properties:

1- Colour:

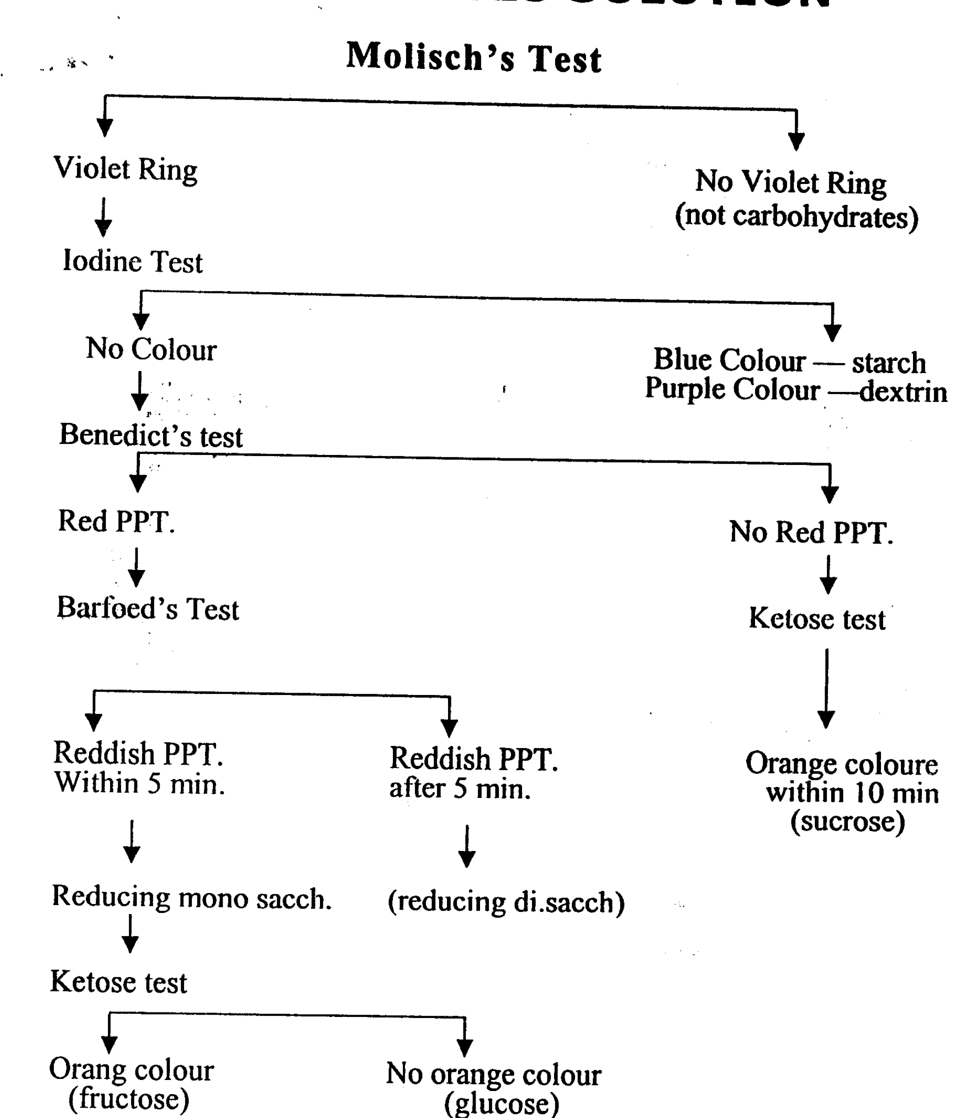
2- Odour:

3- Solubility:

4- Taste:

ii] Chemical properties:

SCHEME FOR UNKNOWN CARBOHYDRATES SOLUTION



Record the results as:

	Test	Observation	Result
1 2 3			

Lipids

Definition :

Are organic substances insoluble in water, but soluble in Fat solvents [Benzene, ether,….], or esters of fatty acids with glycerol.

Composition :

Triacylglycerol (simple lipid) is formed by binding of one molecule of glycerol [Trihydric alcohol] with 3 moles of fatty acids.

Classification :

1) Simple lipids: Esters of glycerol with three fatty acids as triacylglycerol.
2) Conjugated: Are simple lipids conjugated with ph ⟶ ph-lipids, CHO ⟶ glycolipids, or protein ⟶ lipoproteins.
3) Derived: Are derived from above groups such as: fatty acids, steroids, (sterols, steroidal hormones, bile acids), fat soluble vitamins, and carotenoids.

Experiments :

I) Glycerol :

1) Acrolein test :

To on drop of glycerol in a test tube, add 1 ml conc. Sulphuric acid until the mixture fumes which is irritating and pungent (acrolein odour).

Are fatty acids give this test?

No, this test is concerned only with trihydric alcohol (glycerol).

II) Triacylglycerides and fatty acids :

1) Grease stain test :

Is a general test for all lipids. Dissolve small piece of fat in ether, and place one drop on a filter paper and leave to dry, a transparent area is left in site of application denoting presence of lipid.

2) Copper acetate test :

To 3 ml of dissolved lipid in petroleum ether, add 3 ml of copper acetate solution, shake well, leave to stand and record your results:

1- Neutral fat: The two layers remain clear, the upper layer is colourless, while the lower remains blue.
2- Unsaturated fatty acids: The top layer is greenish in colour.
3- Saturated fatty acids: The lower layer shows greenish bluish PPT. while the upper layer is colourless.

****What is the importance of application of Copper acetate test?**

1- Differentiate between neutral fat, sat. F. A and unsat. Fatty acids.
2- Detection of fat rancidity (give +ve test for hydrolysed separated fatty acids).

III) Soaps :

1) Salting out :

To 5 ml of soap solution, add solid Nacl until saturation → the soap is salted out and rises up to the top of solution.

2) Formation of insoluble soap :

To 5 ml of soap solution, add 3-5 drops of 5% calcium chloride ⟶ insoluble white PPT. of calcium soap.

GENERAL SCHEME FOR IDENTIFICATION OF SIMPLE UNKNOWN SOLUTION

I) Physical Properties:

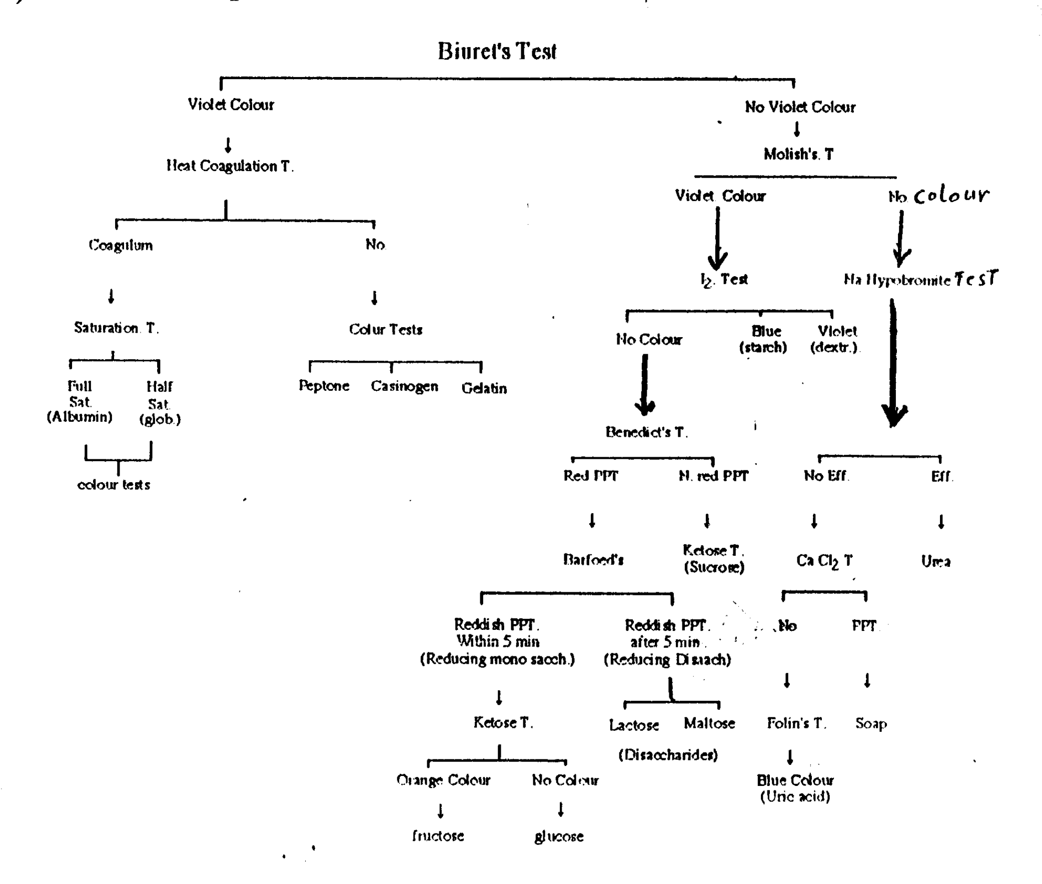
1) Colour: i) colorurless, ii) whitish, iii) yellowish

2) Odour: i) odourless, ii) sp. Odour.

3) Aspect: i) clear, ii) turbid.

4) Reaction (pH): i) acid, ii) alkaline, iii) neutral

II) Chemical Properties:



Record the results as:

Test	Observation	Result

ENZYMES

Definition :

Are protein thermolabile catalyst, accelerate the speed of chemical reaction without being consumed in that reaction, enzymes are produced by living cells.

Importance :

Are essential for all chemical reactions required for continuation of life.

****What are factors affecting rate of the enzymatic reactions?**

pH, temp., enzyme conc., substrate conc., enzyme inhibitors, enzyme activators.

Experiments :

I) Effect of temperature on amylase activity of saliva :

Preparation of Saliva :

- Rinse mouth with little warm water. Then take 10 – 15 ml of warm water and circulate it in mouth by tongue for at least two minutes. Collect in clean beaker, and filter.
- **Prepare two test tubes:**
  - In tube No.1 place 3 ml of saliva + 3 ml of 1% starch solution.
  - In tube No.2 place 3 ml of boiled saliva + 3 ml of 1% starch solution.
  - Place both test tubes in a water bath at 40°C for 10 min.
  - Add to one ml of each tube one drop of dilutr iodine solution, and mix.
  - To another one ml of each tube, carry out Benedict's test to detect any of reduced sugar (maltose).

Record and comment on your results **:**

	Tube No.1 starch + saliva	**Tube No.2 starch + boiled saliva**
Iodine Test		
Benedict's Test		

****What is the effect of salivary amylase on starch hydrolysis ?**

****What is the optimum temperature of amylase activity ?**

2) Effect of pH on activity of salivary amylase :

* **Prepare 3 tubes** :

- In Tube No.1 place 3 ml of saliva solution + 3 ml of 1% starch solution (neutral).
- In tube No.2 place 3 ml of saliva solution + 3 ml 1% starch + one ml of dilute acetic acid to render it acidic.
- In tube No.2 place 3 ml of saliva + 3 ml starch solution + one ml of 10% Na_2Co_3 to render it alkaline.
- Place all tubes in a water bath at 40°C for 10 minutes.
- Carry out Iodine & Benedict's tests and record your results as previous experiment.

****What is the optimum pH of salivary amylase activity ?**

** Effect of salivary amylase on starch:

Starch $\xrightarrow{\text{amylase}}$ amylodextrin ⟶ erythrodextyin ⟶

Achrodextrin ⟶ maltose [reducing disaccharide].

** Optimum temp. of amylase activity ⟶ 37-40°C.

** Optimum pH of amylase activity ⟶ 6.8 – 7.

DEMONSTRATION THE ACTION OF RENNIN ON MILK (MILK CLOTTING)

I) Milk constituent :

Milk contains :

1- Proteins:

(i) Casein: is ph, Protein, present as Ca, casinogenate (80% of Cow milk, 40% of human milk);

(ii) Lact albumin;

(iii) Lactglobulin.

2- Carbohydrate: lactose [5 – 7%] by Lact. B. acidophilus (bacteria) $\xrightarrow{souring}$ Lactic acid.

3- Fat: Mainly triacylglycerol, small amounts of lecithin, sterols, Vit A,D.

4- Vitamines: Rich in Vit A, B_2, Poor in Vit D and C.

5- Minerals: Rich in Ca^{++}, K, ph, but poor in iron and copper.

6- Enzymes: Proteinase, lipase, amylase, lactoperoxidase, and catalase.

II) Experiment :

Prepare a set of six tubes as follows :

* In tube No.1 add 5 ml milk + 1 ml water + 1 ml rennin solution.
* In tube No.2, add 5 ml milk + 1 ml water + 1 ml boiled rennin.
* In tube No.3, add 5 ml milk + 1 ml 0.5% ammonium oxalates + 1 ml rennin.
* In tube No.4, add 5 ml milk + 0.05% 0.5% ammonium oxalates + 0.5% ml 2% $CaCl_2$ + 1 ml rennin.
* In tube No.5, add 5 ml milk + 0.5% ml 2% acetic acid + 1 ml rennin
* In tube No.6, add 5 ml milk + 0.5 ml $NaCo_2$ 3% + 1 ml rennin.
* Incubate all tubes at 40°C for 15 minutes.
* Record your results and comment.

**** What is opt. temp. for rennin action ?** ⟶ 40°C.

**** Are calcium is important for milk clotting ?** ⟶ form insoluble Ca. paracaseinate. [cheese].

**** What is opt. pH for milk clotting ?** ⟶ pH(4).

No. Tube	**1**	**2**	**3**	**4**	**5**	**6**
Milk clot						

**** What are comparisons between milk clot and blood clot ?**

Milk clot

I- Casein protein of milk $\xrightarrow[\text{enzyme}]{\text{Rennin}}$ soluble paracasein + peptone like substance.

II- Soluble paracasein $\xrightarrow{Ca^{++}}$ insoluble Ca-paracaseinate (milk clot; cheese).

Blood clot

I- Plasma prothrombin $\xrightarrow[Ca^{++}]{\text{thrombokinase}}$ active thrombin.

II- Plasma fibrinogen protein $\xrightarrow[\text{thrombin}]{\text{active}}$ fibrin [Blood clot].

URINE

(I) Physical Properties

1) Volume :

i) Normal: 800 ml – 2000 ml / day.

ii) Polyurea:

Physiological: Protein diet (urea), xanthine bevaradge, salty diet cold weather, polydepsia.

Pathological: diabetes mellitus (4 – 5 L/day), diabetes insipidus (6 – 8 L/day), hypertension, salicylates, I.V. saline drip.

iii) Oligurea:

Physiological: Increase muscular exercise, and sweating, in summer.

Pathological: Renal failure.

2) Reaction :

i) Normal: 5 – 7, average 6.2.

ii) ↓ pH in: High protein diet (sulphates & phosphates), ketoacidosis.

iii) ↑ pH in: Intake of citrious fruits, vegetables, and $NaHCo_3$ intake.

3) Colour :

i) Normal: Pale amber yellow due to urochrome..

ii) Reddish (blood), deep brown (jaundice), creamy (pus cells), may red or orange due to food additives or drugs.

4) Odour :

i) Normal: Urinoferous (aroumatic) odour.

ii) Ammonical: Due to effect of microorganisms on urea → ammonia → Turn pH alkaline → PPT. of phosphate → Turbidity of urine.

iii) Putrid odour: due to pus cells.

5] Aspect :

i) Clear: Transparent, or slightly turbid due to epithelial cells.

ii)Turbid: Pinkish (urates), phosphates in (alk. Urine), pus in gonorrhea, RBCs.

6] Deposit :

i) Absent: Normal.

ii)Present: * Inorganic urates, phosphates.

* Organic: Pus cells, RBCs, casts, epithelial shedding.

7] Specific Gravity :

Depends upon dissolved salts in urine (urea, NaCl, uric acid and glucose) by urinometer, with correction according to room temperature.

$$\text{Sp. Gravity} = \text{Reading} + \frac{\text{Room temp.} - 15}{3}.$$

**** What is Long's formula ?**

* If sp. Gravity is 10<u>20</u> : amount of dissolved salts in urine = 20 X 2.66 = 53.2 gm/liter.

(II) Normal Constituents of Urine

A] Organic :

1) Urea:

Source: Exogenous protein diet, formed in liver.

Daily excretion: 20 – 35 gm/day [= 80 – 90% of total urinary nitrogen, but 50% of total urinary solids].

Experiment:

To 5 ml of Alkaline Sodium hypobromite, add ½ ml urine ⟶ bubbles of nitrogen gas (urea).

$H_2N. Co. NH_2 + 3 N.a oB r \longrightarrow 3 Na Br + N_2 + Co_2 + 2 H_2O$

2) Uric Acid:

Source: Purine bases in protein, fish diets, wear & tear of human cells.

Daily excretion: 0.7 – 1 gm/day

Experiment:

To 5 ml of urine, add 1 ml 10% NaoH and 2 ml Folin's reagent $\xrightarrow{shake}$ blue colour

3) Creatinine:

Source: Endogenous from muscular creatine phosphate.

Daily excretion: 1 – 2 gm/day according to muscular mass (20 mg/kg/day).

Normal creatinine clearance: Is more than 100ml/min, it decreased in renal failure.

Experiment:

To 5 ml of urine, add few drops of saturated solution of picric acid and shake, then add few drops of 10% NaoH and mix ⟶ red or orange colour [creatinine picrate].

4) Ammonia:

Source: * 60% from glutamine in kidney $\xrightarrow{glutaminase}$ glutamic acid + ammonia.

* Bacterial action on amino acids in intestine
* Deamination of amino acids in liver (about 40%).

Daily excretion: 0.7 – 1 gm/day.

Experiment:

To 5 ml of urine, add 1 ml 10% NaoH and boil ⟶ ammonia odour, by wet red litmus paper it turns blue.

5) Creatine:

Source: In urine of children, and may in pregnancy.

Daily excretion: 50 – 150 mg/day.

6) Hippuric acid:

Source: Is end product of benzoic acid detoxication in liver, putrifaction of tyrosine & ph. Alanine in intestine..

Daily excretion: 0.7 gm/day.

7) Amino acids (100-200mg/day) and some enzymes as lipase, amylase and urokinase.

** Pathological aminaciduria: occurs in severe liver disease, or inherited defects as phenyl ketonuria, histidinemia and maple syrup urine disease.

B] Inorganic :

1) Chlorides:

Source: Salt of diet.

Daily excretion: 10 – 15 gm/day.

Experiment:

To 5 ml of urine, add ½ ml diluted nitric acid, and 1 ml of silver.

Nitrate solution $\xrightarrow{\text{shake}}$ white curdy PPT (silver chloride).

2) Phosphate:

Source: Phosphoproteins of egg yolk, casein of milk, nucleoproteins, and phospho Lipids.

Daily excretion: 1 – 2 gm/day.

Experiment:

To 5 ml of urine, add 5 ml conc. Nitric acid with caution, and 2 ml saturated ammonium molybdate solution, boil in water bath $\longrightarrow$ yellow PPT (ammoniophosphomolybdate).

3) Sulphates:

Source: Sulpher containing amino acids of proteins.

Daily excretion: 1 – 2 gm/day.

Experiment:

To 5 ml of urine, add 2 ml conc. Nitric acid, and 2 ml barium chloride solution, and mix gently ⟶ white ppt. (insoluble barium sulphate).

4) Carbonate:

* May present in alkaline urine.
* 5 ml urine + few drops conc. Hcl ⟶ efferesence [Co_2].

(III) Pathological Constituents of urine:

1) Albumin :

i) Physiological albuminurea: Stress, severe exercise, long stand up right position (postural albuminurea), high protein diet.

ii) Pathological:

Pre renal: Heart failure, fever, hypertension.

Renal: (True albuminurea), in glomeronephritis, nephrotic syndrome, and renal tumours.

Post renal: Lower urinary tract infection, vaginal discharge.

** ***What is Bence Jones protein ?*** Is low molecular weight light chain γ-globulins, coagulate at 40 – 60C°, re-dissolve at 100C°, occurs in cases of myeloma and myeloid leukemia.

iii) Experiment:

** **Heat coagulation test:**

* Heat gently the upper half of test tube filled with urine ⟶ coagulum.
* Addition of few drops of diluted acetic acid helps coagulation, why? .... ⟶ To attain isoelectric point of albumin (pH 4.7) where max. PPT. occurs.
* Precipitation with sulphosalicylic acid 25%.
* Add few drops of sulphosalicylic acid to urine solution ⟶ opacities (coagulum).

peroxidase

II- H_2O_2 + reduced chromagen ⟶ coloured oxidized chromagen + H_2O.

** The test tape is immersed in urine for 1-2 seconds, read the colour after one minute on the strip?

3] Ketone Bodies :

* Ketone bodies may present in urine (physiological in high fat diet or pathological ketonuria, in diabetic ketoacidosis.

* Types are acetoacetic acid, B. hydroxybutyric acid, and acetone (is volatile excreted in expired air).

* Which is present in Urine?⟶ 2/3 B.hydroxyl butyrate, 1/3 acetoacetate.

Experiment:

1- Rothera's Test: For aceto acetate and acetone.

* To 5 ml of urine, add solid ammonium sulphate until full saturation.
* Add 5 drops of sodium nitroprusside solution and mix.
* Add 3 ml of strong ammonium hydroxide solution and mix.
* Wait up to 10 minutes, if ketone bodies are present a violet colour will develop.
* The strength of colour is proportional to concentration of ketone bodies.

4] Bile Salts :

* Present in urine in case of obstructive jaundice.

Experiment:

**** Hay's sulphur test:**

* Fill dry test tube with filtered urine.
* Sprinkle a little amount of powdered sulphur on surface of urine.
* In normal urine, sulphur floats on surface.
* In presence of bile salts sulphur sinks down, Why? ⟶ Due to decrease of surface tension of urine.

5] Bile Pigments :

* Bilirubin appears in urine in case of obstructive and hepato-toxic jaundice, but not in haemolytic type. Why? Because unconjugated bilirubin (present in hemolytic type) is water insoluble, not exerted in urine.

*** What is the colour of urine containing bile pigments?**

Deep brown with yellow coloured froth.

Experiment:

**** Alcoholic Iodine test:**

* To 5 ml of unfiltered urine, add carefully 10 drops of alcoholic iodine on surface of urine.
* A green ring gradually develops between the two layers, if urine contains bilirubin.

6] Blood :

* Present in urine in cases of urinary stones, nephritis, tumours, trauma.

Experiment:

**** Benzidine test:**

* To a mixture of benzidine reagent and hydrogen peroxide H_2O_2 [1ml + 1ml], add 10 drops of urine, drop by drop ⟶ greenish blue colour.

** ***Principle :***

* RBcs contain enzyme catalase which acts on $2H_2O_2 \rightarrow 2H_2O + O_2$
* Liberated oxygen will oxidize Benzidine reagent ⟶ greenish blue colour.

7] Indication :

* May present in urine in case of intestinal putrifaction (–indole ring of tryptophan).

Experiment:

**** Jaffe's test:**

* To 3 ml of urine in a test tube, add 3 ml of 10% lead acetate, and filter.
* To filtrate add equal volume of conc. HCL + 2 ml chloroform + one drop of K. chlorate solution, shake well, and wait 2 minutes for separation of the layers.
* Blue colour is developed in the top chloroform layer.

IV- Microscopic Examination of Urine

* Urine sediment must be examined (after centrifugation) to give idea about the presence of RBCs (haematourea), pus cells (infections), crystals (stones) or Bilharizal ova.
* One drop of urinary sediment is layered on clean glass slide, and examine for the presence of:

1) Organized sediments:

Pus cells, RBcs, Casts and Bilharizal ova.

2) Unorganized sediments:

i) in acid urine: calcium oxalates, or urates.

ii) in alkaline urine: triple phosphate or ammonium urates.

COMPLETE REPORT ON SAMPLE OF URINE

I) Physical Properties :

1) Volume:
2) Colour:
3) Reaction:
4) Odour:
5) Aspect:
6) Sediments (Deposit):
7) Specific Gravity:

II) Pathological constituents :

Test	Observation	Result
1) Proteins *(Heat coagulation test)* 2) Sugars *(Benedict's or Test Tape).* 3) Ketone Body *(Rothera's Test).* 4) Blood *(Benzidine Test).* 5) Bile Salts *(Hay's Sulphur Test).* 6) Bile Pigments *(Iodine Test).* 7) Indican *(Jaffe's Test).*		

III) Microscopic Examination :

COLORIMETRIC ESTIMATION OF BODY FLUIDS

Colorimotry

Principle :

Many important biological solutions are estimated as coloured solutions, in which the intense of the colour is proportioned to the concentration of that biological substance.

When light pass in a coloured solution, it has specific wave length, therefore, the intense of the colour is measured as its optical density or absorbance of its wave length.

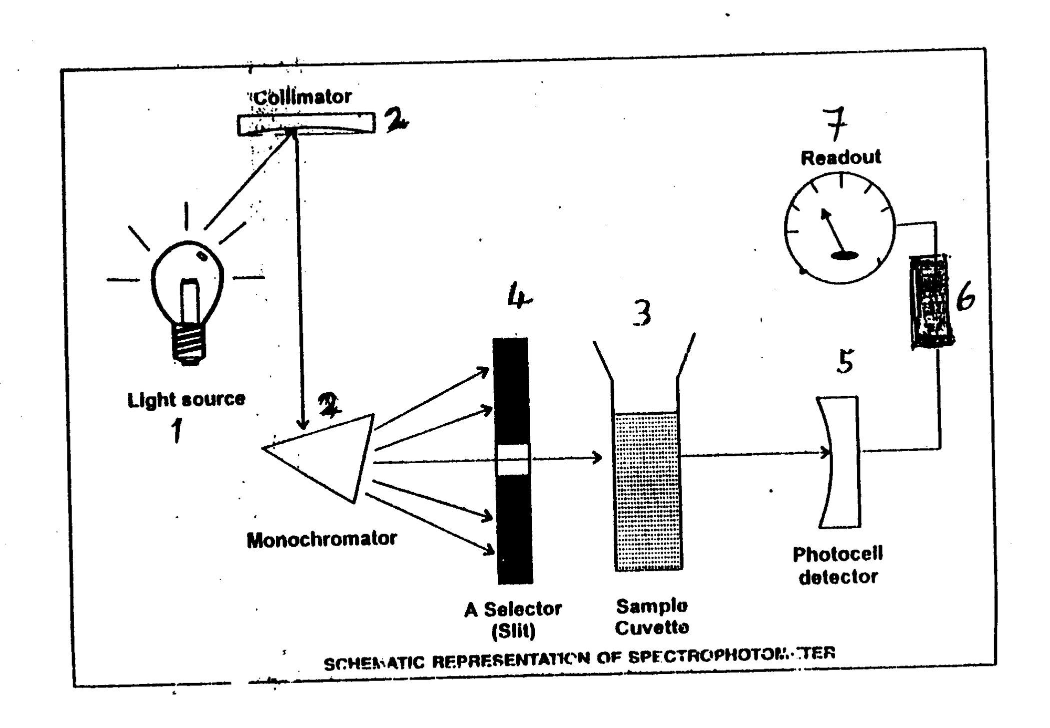
Colorimeter measures the absorbance or optical density of the coloured solution, and the intense of colour is read on the colorimeter scale.

The test unknown solution is compared with a known stander to obtain the exact concentration of the test unknown sample.

$$\text{Conc. Test} = \frac{\text{Reading of Test}}{\text{Reading of stander}} \times \text{Conc. Stander}$$

The zero point of the colorimeter should be adjusted first with colourless solution as water, what is called the blank which is riginal solution for colorimetry to be fixed it zero, then measure the standard and test solutions.

COLORIMETER



SCHEMATIC REPRESENTATION OF SPECTROPHOTOMETER

1- Tungesten lamp.

2- Condenser lens (gives paralleled rays).

3- Cuvette: contains the coloured solution.

4- Filter: its colour, is complement to that of coloured solution to allow maximum absorption or transmission.

5- Photocell: generates electric current proportion to light following on it from the coloured solution according to the intense of its colour.

6- Amplifier: Intenses the current falling on it from the photocell.

7- Galvanometer: contains the scale reading.

DETERMINATION OF GLUCOSE BY O-TOLUIDINE METHOD

Principle :

O. Toluidine is an aromatic amine that reacts with glucose in hot acetic acid to produce a coloured product with a peak absorbance at 630 mm or (Red filter).

Normal Value :

Normal fasting true glucose, enzymatic (65 – 110mg%) is, slightly higher wit other chemical methods.

Elevated in :

Diabetes mellitus, hyperthyroidism, cortical excess, hyperpituitrism.

Decreased in :

Hyperinsulinism, cortical hypofunction, hypopituitrism, over dose of insulin management.

Reagents :

1) O. Toluidine reagent.
2) Glucose Standard 100 mg %.

Procedure :

3 test tubes are labeled as blank, test, and standard.

	Blank	Test (Sample)	Standard
Sample	—	0.2 ml	—
Standard	—	—	0.2 ml
Blank	0.2 ml	—	—
O.Toluidine reagent	5 ml	5 ml	5 ml

* Boil in water bath at 100°C for 10 minutes.
* Cool at room temperature, and read the absorbance at 630mm or read filter against blank.

Calculation :

$$\text{Glucose mg\%} = \frac{\text{Reading of Test}}{\text{Reading of stander}} \times \text{Conc. Standard}$$

*** Record your results and comment.**

Experiment

Date :

Name of Test :

Results :

Comment : 1) Normal: a) Fasting b) Post prandial

2) Hyperglycemia in cases of ....

3) Hypoglycemia in cases of ....

ESTIMATION OF TOTAL SERUM PROTEINS

Principle :

Copper in alkaline solution (blue colour) reacts with peptide bond of amino acids in protein producing a violet colour.

Normal total serum protein :

6 – 8.2 gm% [Alb:3.5 – 5.5 gm%, glob 2 – 3 gm and fibrinogen in plasma 0.2 – 0.4 gm%].

Hyperproteinemia :

Associated with an increase in globulins as infectious hepatitis, myeloma, typhus, malaria.

Hypoproteinemia :

Associated with decrease in albumin as malnutrition, acute & chronic hepatic diseases, nephritis, and leukemia, or decrease in globulins as in malnutrition, congenital or acquired hypogamma globenemia, and lymphatic leukemia.

Reagents :

1) Biuret's reagent.
2) Standard protein solution, 7% gm.

Procedure :

Prepare three tubes as follows.

	Blank	Test	Standard
Dist. Water	0.5 ml	—	—
Sample	—	0.5 ml	—
Standard	—	—	0.5 ml
Biuret reagent	3 ml	3 ml	3 ml

* Read at 540 nm.

Experiment

Date :

Name of Test :

Results :

Comment :

DETERMINATION OF SERUM ALBUMIN BY BROMOCRESOL GREEN

Principle :

Bromocresol green reacts with albumin at acedic PH, producing a greenish yellow colour complex.

Normal value :

3.5 – 5.5 gm%.

Elevated in :

Dehydration, shock, hoemo-concentration.

Decreased in :

Malnutrition, malabsorption syndrome, acute and chronic renal or hepatic diseases, and leukemia.

Reagents :

1) Bromocresol green reagent.
2) Standard albumin 5 mg%.

Procedure :

Prepare 3 tubes as follows.

	Blank	Test	Standard
Sample	—	0.1 ml	—
Standard	—	—	0.1 ml
Water Blank	0.1 ml	—	—
Reagent	3 ml	3 ml	3 ml

* Mix well, incubate at room temperature for 5 minutes, and read at 620mm.

Calculation :

$$\text{Total albumin} = \frac{\text{Reading of Test}}{\text{Reading of standard}} \times \text{Conc. Standard}$$

**** Record your results and comment.***

Experiment

Date :

Name of Test :

Results :

Comment :

DETERMINATION OF CREATININE IN URINE

Principle :

Creatinine reacts with picric acid in alkaline solution to form a red-orange chromagen (picramic acid).

Normal value :

20 – 25 mg/kg/day; about 1 – 1.5gm/day.

Elevated in :

Acute and chronic renal insufficiency, and impairment of renal function induced by some drugs..

Reagents :

1) Saturatd picric acid solution.
2) Sodium hydroxide 0.75. N (3 gm%).
3) Creatinine standard 2 mg%.

Procedure :

Urine is diluted 1 to 20

	Blank	Test	Standard
Sample	—	3 ml	—
Standard	—	—	3 ml
Water Blank	3 ml	—	—
Picric acid	1 ml	1 ml	1 ml
Na oH	1 ml	1 ml	1 ml

* Mix well, leave at room temperature for 10 minutes and read at 520 nm or green blue filter.
* Record your results, and comment.

Calculation :

$$\frac{\text{Test}}{\text{Standard}} \times 20 \times 2 = \qquad \text{mg\%/day}$$

Experiment

Date :

Name of Test :

Results :

Comment :

DETERMINATION OF CREATININE IN SERUM

Reagents :

1) Picric acid 35 mmol/1 solution.
2) Na oH 1.6 N solution (6.4 gm%).
3) Creatinine standard 2 mg%.
4) 10% trichloro acetic acid as precipitate solution.,

Normal value :

0.7 – 1.5 gm%, it depends on the body muscular mass.

Procedure :

* Test :

- Add 1.5 ml of PPT. solution to 1.5 ml of separated serum, mix and centrifuge.
- To one ml of spt. Solution, add 1 ml of picric acid and 1 ml of Na oH solution, and mix.

* Standard :

- Add 1 ml of each picric acid and Na oH solutions to 1 ml of creatinine standard, and mix.

* Blank :

- Add 1 ml of each picric acid and Na oH solutions to 1 ml of dist. Water.
- mix, stand 10 minutes at room temperature, and read at 520nm, or green blue filter.

Calculation :

$$\frac{\text{Test}}{\text{Standard}} \times 2 = \qquad \text{mg\%}$$

Experiment

Date :

Name of Test :

Results :

Comment :

** **Creatinine clearance test:**

- The test denotes the statement of renal function (glomerular filtration rate).
- It depends upon measurement of serum creatinine, and urine creatinine per 24 hour (1440 minutes).
- It calculated by amount of filtered urine by ml per minute, if less than 100ml/min ⟶ renal failure.

$$\text{Creatinine clearance (ml/min)} = \frac{\text{Urinary creatinine X urine volume per/day by ml}}{\text{Plasma creatinine X collection time in day per min (1440)}}$$

DETERMINATION OF TOTAL SERUM CHOLESTEROL

Principle :

Cholesterol form a coloured complex [Cholestahexene sulphonic acid], by reactions with acetic acid (solvent and dehyderating reagent), and Sulphuric acid (dehydrating and oxidizing reagent), which is the base of Lieberman – Burchard reactions.

Normal value :

120 – 220 mg%

Borderline: 240 mg%

Elevated: above 240 mg%

Elevated in :

Diet rich in saturated fatty acids. Familial hypercholesterolemia type II, hypothyrodism, poorly controlled diabetes, nephrotic syndrome, chronic hepatitis, and obstructive jaundice.

Decreased in :-

Acute hepatitis, Gaucher's disease, and hyperthyroidism. Diet rich in polyunsaturated fatty acids and prolonged fasting.

Reagents :

1) Acetic Acid anhydride A.R.
2) Glacial acetic acid A.R.
3) Conc. Sulphuric acid A.R.
4) Standard cholesterol 200 mg% in glacial acetic acid.

Procedure :

Prepare 3 test tubes in cold water bath:

	Blank	Test	Standard
Acetic anhydride	1.5 ml	1.5 ml	1.5 ml
Glacial acetic	1 ml	1 ml	1 ml
Serum	—	0.1 ml	—
Standard	—	—	0.1 ml
Water	0.1 ml	—	—

*** Mix, leave 5 minutes at room teperature**

Cone. H2 So4 [Add in cold water bath, with caution]	0.5 ml	0.5 ml	0.5 ml

* Mix leave 10 minute at room temp., and read at 580 nm, or yellow green filter.

Calculation :

$$\frac{\text{Test}}{\text{Standard}} \times 200 = \qquad \text{mg\%}$$

Experiment

Date :

Name of Test :

Results :

Comment :

DETERMINATION OF SERUM URIC ACID

Principle :

Uric acid in serum reacts with phosphotungestate in alkaline solution producing allantion, which is reduced to a complex blue colour of tungsten.

Normal value :

2 - 7 mg%

Elevated in :

Gout, eclampsia, lekemias, polycythemia, and renal insufficiency.

Decreased in :

Acute hepatitis and treatment with allopurinal.

Reagents :

1) Na tungestate 10%.
2) 2/3 N. Sulphuric acid.
3) Sodium carbonate 14%.
4) Phospotugestate folin reagent.
5) Standard uric acid.

Procedure :

1) In a centrifuge test tube, add 3.5 ml water, 0.5 ml serum sample, 0.5 ml sodium tungestate 10%, and 0.5 ml 2/3 N.H?So4, mix gently and centrifuge.

2) Prepare 3 test tubes as follows:

	Blank	Test	Standard
SPT; of sample	—	3	—
Standard	—	—	3
Water	3	—	—
Sodium carbonate	0.5	0.5	0.5
Folin reagent	0.5	0.5	0.5

* Mix, leave at room temperature for 30 min., read using red filter, or at 700nm

Calculation :

$$\frac{\text{Test}}{\text{Standard}} \times \text{conc. Standard} = \qquad \text{mg\%}$$

Experiment

Date :

Name of Test :

Results :

Comment :

SEPARATION METHODS

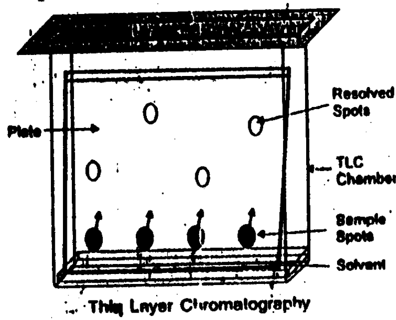
I) Thin layer chimatographyy.

II) Electrophoresis

I- Detection of free amino acids by ascending thin layer chromatography

Concept:

Silica gel precoated sheets are used, they give greater speed and sensitivity than paper chromatography, with sharper separation.



Thin Layer Chromatography

Equipments:

1) - Chromatography glass chamber (tank).
   - Capillary pipettes for spotting samples.
   - Hot oven for drying of sheets.
2) Solvent for amino acids separation:
   N. butanol, glacial acetic acid, and water in ratio of 12:3:5 (v/v).



3) Locating reagent:
   0.3 gm ninhydrin in 100 ml N. butanol, plus 3 ml glacial acetic acid.
4) Amino acid standards:
   Amino acid standards are prepared in 10% isopropyl alcohol in water.

Method:

1) Samples of amino acid standards and test are spotted on silica gel sheet using the capillary tubes.
2) Spots are allowed to dry at room temperature.
3) The sheet is incorporated into the tank, containing the solvent for about 2 hours.
4) The sheet is removed from the tank and dried in the hot oven at 80°C.

5) The dried sheet is sprayed with the locating reagent, and dried again in the oven (110°C/5 min) for developing of the colours of separated amino acids.

6) Test amino acids (samples) are identified by comparing with Standard of amino acids mixture.

Clinical uses:

The procedure is used for detection of abnormal amino acids in serum, urine, saliva or semen and in inborn error of protein metabolism.

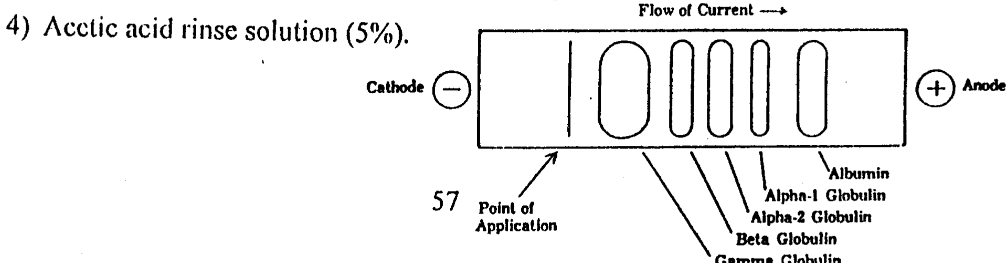
II- Electrophonesis of serum proteins

Concept:

- Fractions of serum protein can be identified quantitavely and qualitavely by electrophoresis.
- Analysis of serum protein fractions aids in diagnosis of various diseases.
- Serum proteins are made up of amino acids which contain both carboxyl [–cooH] and amino [–NH_2] group. By selection of buffer pH, the serum proteins are charged with positive or negative charges.
- The charged serum proteins migrate toward the opposited electrode; if +ve charge molecules move to cathode (if use acid buffer).
- But if –ve charge molecules as in alkaline buffer, they move to anode.

Equipments:

1) Tank for electrophoresis, and cellulose acetate strips as media for application and separation of serum proteins.
2) Alkaline buffer: (pH 8.8) as tris-barbital-sodium barbital buffer;; pH 8.8.
3) Staining solutiuon: 0.5% poncea's S in 7.5% aqueous Trichloro acetic acid.
4) Acetic acid rinse solution (5%).



Procedure :

- Submerge the cellulose acetate strip in Na. barbital buffer for at least 10 minutes after adding 0.5 ml ethanol to speed wetting of strip.
- Remove the strip from the buffer, and lay it flat on an absorbent Whatman filter paper to absorb excess buffer.
- Apply equal amounts of serum samples and control to the strip using capillary tubes.
- Place the strip in the electrophoresis tank containing the Na. barbital buffer.
- Turn on power supply at 250 volt/20 min.
- After end of run time shut off the power and carefully remove the strip, then float it on the surface of ponceau S staining solution for about 10 min.
- Remove the strip and place in the rinsing solution (acetic acid 5%) for about 10 min. with agitating each time to remove excess stain.
- Now, the serum sample is separated into its fractions: albumin, alpha globulins, beta globulin, and gamma globulins.
- If plasma is used additional fibrinogen band is seen.
- The serum bands can be determined quantitavely or qualitavely using the densitometer.
- Variations in serum protein bands can be used in identification of various clinical diseases.

SERUM PROTEIN PATTERNS AND POSSIBLE CAUSES

PATTERN TYPE	TOTAL PROTEIN	ALBUMIN	α_1	α_2	β	γ	A/G
Normal Serum Protein (Approximate ranges)	6-8 g/100 ml	50-65%	2-6%	6-17%	7.5-18%	9-20	1-2
Alpha 2 (acute inflammatory)	N	N	N	↑	N	N	N
Gamma 2, Albumin (Viral Hepatitis)	↓	↓	N	N	N	↑	↓
Broad Gamma (Hepatic Cirrhosis)	↓	↓	N	N	N	↑	↓
High Alpha 2/Gamma Ratio (Nephrotic Syndrome)	↓	↓	N	↑	N	↓	↓
High Alpha 1 & 2; Low Albumin (Malignant Tumor)	↓	↓	↑	↑	N	N	↓
Monoclonol Globulin (Myeloma-Macroglobulinemia)	↑	N, ↓	N	N, ↑*	N, ↑*	↑*	↓
Polyclonal Gammopathies							
1. Infectious:							
Viral Hepatitis	N, ↑	N, ↓	N	N	↓	↑	↓, N
Mononucleosis	N, ↑	↓	N	N	N	↑	↓, N
Bacterial Tuberculosis	N, ↑	N, ↓	N	N, ↑	N	↑	↓, N
Actinomycosis	N, ↑	N, ↓	N	N	N	↑	↓, N
2. Malignancy:							
Monocytic Leukemia	↑	N, ↓	N	↑	N	↑	↓
Hodgkins	↑	N, ↓	N	↑	N	↑	↓
Epithelial with Metastases	↑	N, ↓	↑	↑	N	↑	↓
3. Autoimmune:							
Cirrhosis	N, ↑	↓	N	N	Fusion With Gamma	↑	↓, N
Hemolytic Anemia	N, ↑	N, ↓	N	N, ↓	N	↑	N, ↓
Rheumatoid Arthritis	N, ↑	↓	↑	↑	N	↑	↓
Lupus erythematosis	↑	↓	↑	↑	N	↑	↓
Fraction Deficiency (hypogammaglobulinemia)	↓	N	N	N	N	–	↑
Multiple Myeloma**	↑	↓	N	N	N, ↑	N, ↑	↓

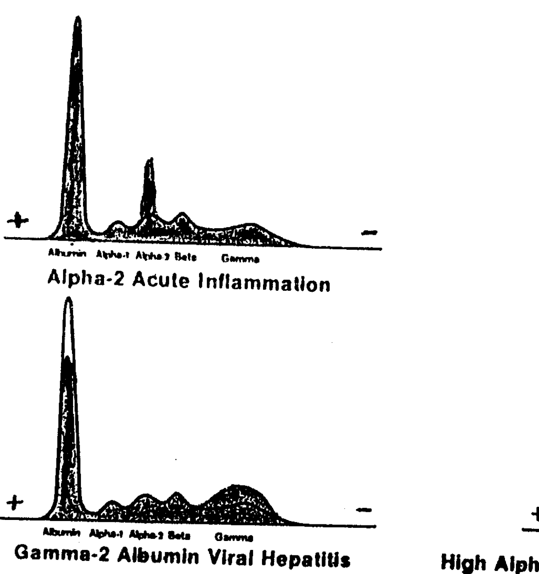
N = Normal ↓ = Decrease
↑ = Increase – = Absent
A/G = Albumin to Globulin Ratio

* Monoclonal band may be seen in any one of these fractions.
** A prominent narrow band will occur somewhere in the β - γ region.

MAJOR SERUM PROTEIN ELECTROPHORETIC PATTERNS

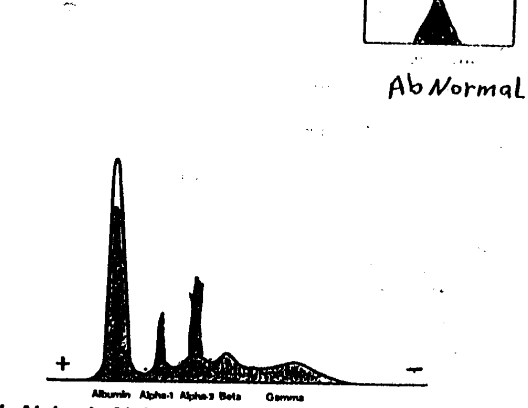
Normal

AbNormal

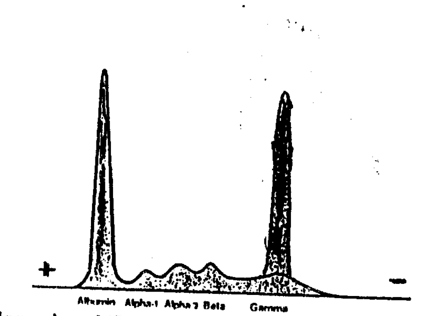


Alpha-2 Acute Inflammation

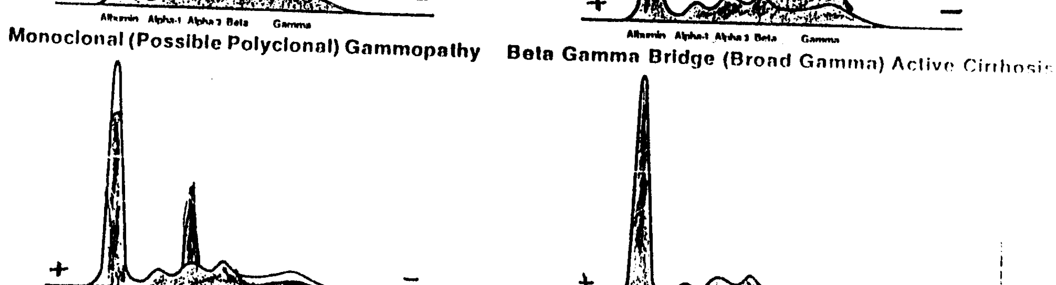
Gamma-2 Albumin Viral Hepatitis



High Alpha-1, Alpha-2 Low Albumin Malignant Tumor



Monoclonal (Possible Polyclonal) Gammopathy



Beta Gamma Bridge (Broad Gamma) Active Cirrhosis

High Alpha-2 Gamma Ratio (Nephrotic Syndrome)

Fraction Deficiency (Congenital)
Hypogammaglobulinemia

Common clinical values in diagnosis

Amylase (Serum):

Normal (varies with method) 80-180 Somogyi units/ 100 ml serum. (One Somogyi unit equals amount of enzyme which will produce 1 mg of reducing sugar from starch at pH 7.2) 0.8-3.2 1U/liter.

A- **Precautions**: If storage for more than 1 hours necessary, blood or serum must be refrigerated.

B- **Physiological basis**: Normally, small amounts of amylase (diastase), molecular weight about 50,000 originating in the pancreas and salivary glands, are presenting the blood. Inflammatory disease of these glands or obstruction of their ducts results in regurgitation of large amounts into enzyme into the blood and increased excretion via the kidney.

C- **Interpretation:**

1- **Elevated in**: acute pancreatitis, obstruction of pancreatic ducts (carcinoma, stone, structure, duct sphincter spasm after morphine), mumps, occasionally in the presence of renal insufficiency, occasionally in diabetic acidosis, and occasionally with inflammation of the pancreas from a perforating peptic ulcer. Rarely, combination of amylase with an immunoglobulin produces elevated serum amylase activity (macro amylase) because the large molecular complex (molecular weight at least 160,000) is not filtered by the glomerulus's

2- **Decreased in**: hepatitis, acute and chronic; pancreatic insufficienc, and occasionally in toxemia of pregnancy.

D- **Drugs Effects on laboratory result**: Elevated by morphine, codeine, merperidine, methacholine, pancreozymin, sodium diatrizoate, and cyproheptadine. Pancreatitis may be induced by indomethacin, furosemide, chlorthalidone, coticosteroids, histamine, salicylate, and tetracycline. Decreased by barbiturate poisoning.

Amylase, urine:

Normal: varies with method. 40-250-somogyi units/hour.

A- **Precautions**: If the determination is delayed more than 1 hour after collecting the specimen, urine must be refrigerated.

B- **Physiologic basis**: See Amylase, serum. If renal function is adequate, amylase is rapidly excreted in the urine. A timed urine specimen (ie, 2,6, or 24 hours) should be collected and the rate of excretion determined

C- **Interpretation**: Elevation of the concentration of amylase in the urine occurs in the same situations in which serum amylase concentration is elevated. Urinary amylase concentration remains elevated for up to 7 days after serum amylase levels have returned to normal following an attack of pancreatitis. Thus the determination of urinary amylase may be useful if the patient is seen late in the course of an attack of pancreatitis. An elevated serum amylase with normal or low urine amylase excretory rate may be seen in the presence of renal insufficiency.

Bilirubin, serum:

Normal: Total 0.2-1.2 mg/100ml (SI: 3.5-20.5 μmol/l). Direct (glucuronide). 0.1-0.4-mg/100 ml. Indirect (unconjugated), 0.2-0.7 mg/100ml. (SI: direct, up to 7 μmol/l; indirect, up to 12 μmol/l.)

A- **Precautions**: The fasting state is preferred to avoid turbidity of serum. For optimal stability of stored serum, samples should be frozen and stored in the dark.

B- **Physiological Basis**: Destruction of hemoglobin yields bilirubin, which is conjugated in the liver to the diglucuronide and excreted in the bile. Bilirubin accumulates in the plasma when near liver insufficiency exists, biliary obstruction is present, or the rate of hemolysis increases. Rarely, abnormalities of enzyme systems involved in bilirubin metabolism in the liver (eg, absence of glucuronyl transferase) result in abnormal bilirubin concentrations.

C- **Interpretation:**

1- Direct and indirect forms of serum bilirubin are elevated in acute or chronic hepatitis, biliary tract obstruction (Cholangiolar, hepatic, or common ducts). Toxic reactions to many drugs, chemical, and toxins, and Dubin-johnson and Rotor's syndromes.

2- Indirect serum bilirubin is elevated in hemolytic diseases or reactions or reactions and absence or deficiency of glucuronyl transferase, as in Gilbert's disease and Crigler-Najjar syndrome.

3- Direct and total bilirubin can be significantly elevated in normal and jaundiced subjects by fasting 24-48 hours (in some instances even 12 hours) or by prolonged caloric restriction.

D- **Drug Effects on Laboratory Results**: Elevated by acetaminophen, chlordiazepoxide, novobiocin, and acetohexamide. Many drugs produce impairment of liver function.

Calcium, Serum:

Normal: 8.5-10.5 mg/100ml or 4.2-5.2 mEq/liter. (Ionized. 4.2-5.2 mg/100ml or 2.1-2.6 mEq/liter)(SI: total. 2.1-2.6 mmol/l; ionized, 1.05-1.3 mmol/l)

A- Precautions: Glassware must be free of calcium. The patient should be fasting. Serum should be promptly separated from the clot.

B- Physiologic Basis: Endocrine, renal, gastrointestinal, and nutritional factors normally provide for precise regulation of calcium concentration in plasma and other body fluids. Since some calcium is bound to plasma protein, especially albumin, determination of the plasma albumin concentration is necessary before the clinical significance of abnormal serum calcium levels can be interpreted accurately.

C- Interpretation:

1-Elevated in hyperparathyroidism, secretion of parathyroid-like hormone by malignant tumors, vitamin D excess, milk-alkali syndrome, osteolytic disease such as multiple myeloma, invasion of bone by metastic cancer; Paget's disease of bone, Boecks's sarcoid, and immobilization. Occasionally elevated with hyperthyroidism and with ingestion of thiazide drugs.

2-Decreased in hypoparathyroidism. Vitamin D deficiency (rickets, osteomalacia), renal insufficiency, hypoproteinemia, malabsorption syndrome (sprue, ileitis, celiac disease, pancreatic insufficiency), severe pancreatitis with pancreatic necrosis, and pseudohypoparathyroidism.

Calcium. Urine:

Daily Excretion: Ordinarily there is a moderate continuous urinary calcium excretion of 50-150 mg/24 hours, depending upon the intake. (SI: 1.2-3.7 mmol/24 hours.)

A- Procedure: The patient should remain on a diet free of milk or cheese for 3 days prior to testing; for quantative testing, a neutral ash diet containing about 150 mg calcium per day is given for 3 days. Quantative calcium secretion studies may be made on a carefully timed 24-hours urine specimen. The screening procedure with the Sulkowitch reagent is simple and useful.

B- Interpretation: On the quantative diet a normal person excretes 125 ± 50 mg (1.8-4.4) of calcium per 24 hours. Normally, a slight (1+) cloud reaction (Sulkowitch) occurs if milk and cheese are not present in the diet. In hyperparathyroidism, the urinary calcium excretion usually exceeds 200 mg/24 hours (5 mmol/day). Urinary calcium excretion is almost always elevated when serum calcium is high.

Cholesterol, Plasma or Serum:

Normal: 150-280 mg/100 ml. (SI: 3.9- 7.2 mmol/l)

A- Precautions: The fasting state is preferred

B- Physiological Basis: Cholesterol concentrations are determined by metabolic functions, which are influenced by heredity, nutrition, endocrine function, and integrity of vital organs such as the liver and kidney. Cholesterol metabolism is intimately associated with lipid metabolism.

C- Interpretation:

1- Elevated in: familial hypercholesterolemia (xanthomatosis), hypothyroidism, poorly controlled diabetes mellitus, nephritic syndrome, chronic hepatitis, biliary cirrhosis, obstructive jaundice, hypoproteinemia (idiopathic, with nephrosis or chronic hepatitis), and lipidemia (idiopathic, familial).

2- Decreased in: acute hepatitis and Gaucher's disease, occasionally in hyperthyroidism, acute infections, anemia, and malnutrition.

D- Drug effects on laboratory results: Elevated by bromides, anabolic agents, trimethadione, and oral contraceptive. Decreased by cholestyramine resin, haloperidol, nicotinic acid, salicylates, thyroid hormone, estrogens, clofibrate, chlorpropamide, phenformin, kanamycin, neomycin, and phenyramidol.

Cholesterol Esters, Plasma or serum:

Normal: 65-75 of total serum or plasma cholesterol.

A- Precautions: none

B- Physiologic Basis: Cholesterol is esterified in the intestinal mucosa and in the liver. Cholesterol exists in plasma or serum as the free form (25-33% of total) and as the ester (67-75% of total). In the presence of acute hepatic insufficiency (as in acute hepatitis.) the concentration of esters is reduced.

C- Interpretation:

1- Elevated along with cholesterol in absence of hyperbilirubinemia (see cholesterol, above). The ratio of ester/total cholesterol under these circumstances is normal. With hyperbilirubinemia, absolute values may be elevated, but not in the same proportion as total cholesterol, so that the ester/total cholesterol ratio is less than 65.

2- Decreased in acute hepatitis. Cholesterol esters maybe decreased also in chronic hepatitis and chronic biliary obstruction: in these situations the decrease in total cholesterol. Which results in ester/total cholesterol ratio of less than 65.

Creatine phosphokinase (CPK). Serum:

Normal: varies with method. 10-50 IU/liter

A- Precautions: The enzyme is unstable, and the red cell content inhibits enzyme activity. Serum must be removed from the clot promptly. If assay cannot be done soon after drawing blood, serum must be frozen

B- Physiologic basis: CPK splits creatine phosphate in the presence of ADP to yield +ATP, skeletal and heart muscle and brain are rich in the enzyme.

C- Interpretation: normal values vary with the method

1- Elevated in the presence of muscle damage such as with myocardial infarction, trauma to muscle, muscular dystrophies, polymyositis, severe muscular exertion, hypothyroidism, and cerebral infraction (necrosis). Following myocardial infarction, serum CPK concentration increases rapidly (within 3-5 hours). And remains elevated for a shorter time after the episode (2 or 3 days) than does GOT or LDH.

2- Not elevated in pulmonary infarction or parenchymal liver disease.

Creatine phosphokinase Isoenzymes, serum:

A- Precautions: As for CPK above

B- Physiological basis: CPK consists of 3 proteins separable by electrophoresis. Skeletal muscle is characterized by isoenzyme MM, myocardiaum by isoenzyme MB, and brain by isoenzyme BB.

C- Interpretation: When CPK is elevated because of myocardial damage, there may be an increase in fraction 2, MB. If there is trauma to skeletal muscle or heavy physical exertion, fraction 3. MM will be elevated. A large amount of fraction 1. BB may appear following brain damage. Experience has not been extensive enough to define the specificity of changes in various diseases.

Serum creatine pohosphokinase isoenzymes

Isoenzyme	Normal levels
(Fastest) Fraction 1, BB	Absent to small amount
Fraction 2, MB	Absent to trace
(Slowest) Fraction 3, MM	Major fraction

Urine creatine and creatinine normal values (24 hours). *

	Creatine	Creatinine
Newborn	4.5 mg/kg	10 mg/kg
1-7 months	8.1 mg/kg	12.8 mg/kg
2-3 years	7.9 mg/kg	12.1 mg/kg
4-4$^{1/2}$ years	4.5 mg/kg	14.6 mg/kg
9-9$^{1/2}$ years	2.5 mg/kg	18.1 mg/kg
11-14 years	2.7 mg/kg	20.1 mg/kg
Adult male	0-50 mg	25 mg/kg
Adult female	0-100 mg	21 mg/kg

*SI factors: creatine, mg/24 hours X 7.63 = μmol/24 hours; creatinine, mg/24 hours X 8.84 = μmol/24 hours

Creatine, Urine (24 hours): Normal: see table

A- Precautions: Collection of the 24-hour specimen must be accurate. The specimen may be refrigerated or preserved with 10 ml of toluene or 10ml of 5% thymol in chloroform

B- Physiologic basis: creatine is an important constituent of muscle, brain, and blood: in the form of creatine phosphate it serves as a source of high-energy phosphate. Normally, small amounts of creatine are excreted in urine, but in states of elevated catabolism and in the presence of muscular dystrophies, the rate of excretion is increased.

C- **Interpretation:**

1- Elevated in: muscular dystrophies such as progressive muscular dystrophy, myotonia atrophica, and myasthenia gravis; muscle wasting, as in acute poliomyelitis, amyotrophic lateral sclerosis, and myositis manifested by muscle wasting; starvation and cachectic states, hyperthyroidism, and febrile diseases

2- Decreased in: hypothyroidism, amyotonia congenital, and renal insufficiency.

Creatinine, Plasma or serum:

Normal: 0.7-1.5 mg/100 ml. (SI: 60-130 μmol/l)

A- Precautions: Other materials than creatinine may react to give falsely high results.

B- Physiologic Basis: Creatinine, which is derived from creatine, is excreted through the glomeruli of the kidney. Endogenous creatinine is apparently not excreted by renal tubules. Retention of creatinine clearance closely approximates the insulin clearance and is an acceptable measure of filtration rate.

C- Interpretation: Creatinine is elevated in acute or chronic renal insufficiency, urinary tract obstruction, and impairment of renal function induced by some drugs. Values of less than 0.7 mg/100 ml are of no known significance.

D- Drug effects: on laboratory results: Elevated by ascorbic acid, barbiturates, sulfobromophthalein, methyldopa, and phenolsulfonphthalein, all of which interference with the determination by the alkaline picrate method (Jaffe reaction).

Creatine, Urine: see table for normal values.

Glucose, plasma, serum:

Normal: Fasting "true" glucose, 65-110 mg/100 ml. (SI: 3.6-6.1 mmol/l). Because of the difference in glucose concentration in erythrocytes and plasma, whole blood concentrations will vary depending on the hematocrit.

A- Precautions: If determination is delayed beyond 1 hour, Sodium fluoride, about 3-mg/ml bloods, should be added to the specimen. The filtrates may be refregirated for up to 24 hours. Errors in interpretation may occur if the patient has eaten sugar or received glucose solution parenterally just prior to the collection of what is thought to be a fasting specimen. Determination of serum or plasma concentration is preferred over whole blood.

B- Physiologic Basis: The glucose concentration in extra cellular fluid is normally closely regulated, with the result that a source of energy is available to tissues and no glucose is excreted in the urine. Hyperglycemia and hypoglycemia are nonspecific signs of abnormal glucose metabolism.

C- Interpretation

1- Elevated in diabetes mellitus, hyperthyroidism adrenocortical hyperactivity (cortical excess). Hyperpituitarism, and hepatic disease (occasionally).

2- Decreased in hyperinsulinism, adrenal insufficiency, hypopituitarism, hepatic insufficiency (occasionally), functional hypoglycemia, and by hypoglycemic agents

D- Drug effects: on Laboratory Results: Elevated by corticosteroids, chlorthalidone, thiazide diuretics, furosemide, ethacrynic acid, triamterene, indomethacin, oral contraceptives (estrogen-progestin combinations). Isoniazid, nicotinic acid (large doses), phenothiazines, and paraldehyde. Decreased by acetaminophen, phenacetin, cyproheatdine, pargyline, and propranolol.

Potassium, Serum or Plasma:

Normal: 3.5-5 mEq/liter: 14-20 mg/100ml. (SI: 3.5-5 mmol/l).

A- Precautions: Avoid hemolysis, which releases erythrocyte potassium. Serum must be separated promptly from the clot or plasma from the red cell mass to prevent erythrocyte potassium loss.

B- Physiologic Basis: Potassium concentration in plasma determines the state of neuromuscular and muscular irritability. Elevated or decreased concentration of potassium impairs the capability of muscle tissue to contract.

C- Interpretation:

1- Increased in renal insufficiency (especially in the presence of increased rate of protein or tissue breakdown); adrenal insufficiency; and too rapid administration of potassium salts, especially intravenously and with spironolactone.

2- Decreased in:

a- Inadequate intake (starvation)

b- Inadequate absorption or unusual enteric losses- vomiting, diarrhea, or malabsorption syndrome.

c- Unusual renal loss- Secondary to hyperadrenocorticism (especially hyperaldosteonism) and toadrenocorticosteroid therapy, metabolic alkalosis, use of diuretics such as chlorothiazide and its derivatives and the mercurials, and renal tubular acidosis, testosterone administration.

D-Drug Effects: on Laboratory Results: Elevated by triamterene, phenformin. Decreased by degraded tetracycline, phenothiazines, and sodium polystyrenesulfonate resin.

Proteins, Serum or plasma (Includes fibrinogen):

A- Precautions: Serum or plasma must be free of hemolysis. Since fibrinogen is removed in the process of coagulation of the blood, fibrinogen determinations cannot be done on serum.

B- Physiologic Basis: Concentration of protein determines colloidal osmotic pressure of plasma. The concentration of protein in plasma is influenced by the nutritional state, hepatic function, renal function, occurrences of disease such as multiple myeloma, and metabolic errors. Variations in the fractions of plasma proteins may signify specific disease.

C- Interpretation:

1- Total protein, serum-Normal: 6-8 gm/100 ml. (SI: 60-80 g/l) see albumin and globulin fractions, below.

2- Albumin, serum or plasma-Normal: 3.5-5.5 gm/100 ml. (SI: 35-55 g/l)

a) Elevated in dehydration, shock, and hemoconcentration, administration of large quantities of concentrated albumin "solution" intravenously.

b) Decreased in malnutrition, malabsorption syndrome, acute or chronic hepatic insufficiency, neoplastic diseases and leukemia.

3- **Globulin, serum or plasma:**

Normal: 2-3 gm/100 ml. (SI: 20-30 g/l)

a- Elevated in hepatic disease, infectious hepatitis, cirrhosis of the liver, biliary cirrhosis, and hemochromatosis: disseminated lupus erythematosus; acute or chronic infectious diseases, particularly lymphogranuloma venereum, typhus, leishmanasis, schistosomiasis, and malaria; multiple myeloma; and Boeck's sarcoid.

b- Decreased in malnutrition, congenital agammaglobulinemia, acquired hypogammaglobulinemia, and lymphatic leukemia.

4- **Fibrinogen, Plasma:**

Normal: 0.2-0.6 gm/100ml. (SI: 5.9-17.6 μmol/l.)

a- Elevated in glomerulonephritis, nephrosis (occasionally), and infectious disease.

b- Decreased in disseminated intravascular coagulation (accidents of pregnancy including placental ablation, amniotic fluid embolism, violent labor, meningococcal meningitis, metastic carcinoma of the prostate and occasionally of other organs, and leukemia), acute and chronic hepatic insufficiency, and congenital fibrinogenopenia.

Serum protein fractions are determined by electrophoresis.

	Percentage of total protein
Albumin	52-68
α_1 globulin	2.4-4.4
α_2 globulin	6.1-10.1
β globulin	8.5-14.5
γ globulin	10-21

Serum gamma globulins by immunoelectrophoresis.

IgA	90-450 mg/100 ml
IgG	700-1500 mg/100 ml
IgM	40-250 mg/100 ml
IgD	0.3-40 mg/100 ml
IgE	0.006-0.16 mg/100 ml

Table 12. Some constituents of globulins.

Globulin	Representative constituents
α_1	Thyroxin-binding globulin Transcortin Glycoprotien Lipoprotein Antitrypsin
α_2	Haptoglobin Glycoprotien Macroglobulin Cerulopasmin
β	Transferring Lipoprotein Glycoprotien
γ	γG γD γM γE γA

Triglycrides, Serum:

Normal: < 165 mg/100 ml. (SI: <1.65 g/L)

A- Precautions: Subject must be in a fasting state (preferably for at least 16 hours). The determination may be delayed if the serum is promptly separated from the clot and refrigerated.

B- Physiologic Basis: Dietary fat is hydrolyzed in the small intestine, absorbed and resynthesized by the mucosal cells, and secreted into lacteals in the form of chlyomicrons. Triglycrides in the chylomicrons are cleared from the blood by tissue lipoprotein lipase (mainly adipose tissue) and the split products absorbed and stored. Free fatty acids derived mainly from adipose tissue are precursors of the endogenous triglycrides is in associations with β lipoproteins, the very low-density lipoproteins. In order to assure measurement of endogenous triglycrides, blood must be drawn in the postabsorptive state.

C- Interpretation: Concentration of triglycrides, cholesterol, and lipoproteins fractions (very low density, low density, and high density) is collectively. Disturbances in normal relationships of these lipid moieties may be primary or secondary.

1- Elevated (hyperlipoproteinemia):

a- Primary- type I hyperlipoproteinemia (exogenous hyperlipidemia). Type II hyperbetalipoproteinemia, type III broad beta hyper-lipoproteinemia. Type IV hyperlipoproteinemia (exogenous hyper-lipidemia). And type V hyperlipoproteinemia (mixed hyper-lipidemia).

b- Secondary- Hypothyroidism, diabetes mellitus, nephritic syndrome, chronic alcoholism with fatty liver, ingestion of contraceptive steroids, biliary obstruction, stress.

2- Decreased (hypolipoproteinemia):

a- Primary- Tangier disease (α-lipoprotein deficiency), abetalipopro-teinemia, and a few rare, poorly defined syndromes.

b- Secondary- Malnutrition, malasorption, and occasionally with parenchymal liver disease.

Urea nitrogen & Urea, Blood, Plasma, or Serum:

Normal: BUN, 8-25 mg/100 ml (SI: 2.9-8.9 mmol/l). Urea, 21-53-mg/100 ml (SI: 3.5-9 mmol/l)

A- Precautions: *Do not use* ammonium oxalate or "double oxalate" as anticoagulant, for the ammonia will be measured as urea. Do not use too much oxalate, for it will impair urease activity.

B- Physiologic Basis: the kidney excretes Urea, an end product of protein metabolism. In the glomeular filtrate the urea concentration is the same as in the plasma. Tubular reabsorption of urea varies inversely with rate of urine flow. Thus urea varies inversely with rate of urine flow. Thus urea is a less useful measure of glomerular filtration than is creatinine, which is not reabsorbed. BUN varies directly with protein intake and inversely with the rate of excretion of urea.

C- **Interpretation:**

1- Elevated in-

a- Renal insufficiency- Nephritis, acute and chronic; acute renal failure (tubular necrosis), urinary tract obstruction.

b- Increased nitrogen metabolism associated with diminished renal blood flow or impaired renal function- Dehydration from any cause, gastrointestinal bleeding (combination of increased protein from the digestion of blood plus decreased renal blood flow).

c- Decreased renal blood flow-Shock, adrenal insufficiency, occasionally congestive heart failure, nephrosis not complicated by renal insufficiency, and cachexia.

D- Drug effects on Laboratory results: Elevated by many antibiotics that impair renal function, guanethidine, methyldopa, indomethacin, isoniazed, propanolol, and potent diuretics (decreased blood volume and renal blood flow).

Uric acid, serum or plasma:

Normal: Males, 3-8 mg/100 ml (SI: 0.18-0.48 mmol/l); females, 1.5-6 mg/100 ml (SI: 0.09-0.36 mmol/l).

A- Precautions: If plasma is used, lithium oxalate should be used as the anticoagulant; potassium oxalate may interfere with the determination.

B- Physiologic Basis: Uric acid, an end-product of nucleoprotein metabolism, is excreted by the kidney Gout, a genetically transmitted metabolic error, is characterized by an increased plasma or serum uric acid concentration, an increase in total body uric acid, and deposition of uric acid in tissues. An increase in uric acid concentration in plasma and serum may accompany increased nucleoprotein catabolism (blood dyscrasias', therapy with antileukimic drugs), thiazide diuretics, or decreased renal excretion.

C- **Interpretation:**

1- Elevated in gout, toxemia of pregnancy (eclampsia), leukemia, polycythemia, therapy with antileukemic drugs and a variety of other agents, renal insufficiency, glycogen storage disease (type I), Lesch-Nyhan syndrome (X linked hypoxanthine-guanine phosphoribosyl transferase deficit), and Down's syndrome. The incidence of hyperuricemia is greater in Filipinos than in whites.

2- Decreased in acute hepatitis (occasionally), treatment with allopurinol, probenecid.

D- Drug effects on laboratory results: Elevated by salicylates (low doses), thiazide diuretics, ethacrynic acid, spironolactone, furosemide, triamterene, and ascorbic acid. Decreased by salicylates (large doses), methyldopa, and clofibrate. Phenylbutazone, cinchophen, sulfinpyrazone, and phenothiazines.

Uric acid, Urine:

Normal: 350-600 mg/24 hours on a standard purine-free diet. (SI: 2.1-3.6 mmol/24 hours.) Normal urinary uric acid/ creatinine ratio for adults is 0.21-059; maximum of 0.75 for 24-hour urine while on purine-free diet.

A- Precautions: Diet should be free of high-purine foods prior to and during 24-hour urine collection. Strenuous activity may be associated with elevated purine excretion.

B- Physiologic Basis: Elevated serum uric acid may result from overproduction or diminished excretion.

C- **Interpretation:**

1- Elevated renal excretion occurs in about 25-30% of cases of gout due to increased purine synthesis. Excess uric acid synthesis and excretion are associated with myeloproliferative disorders. Lesch-Nyhan syndrome (hypoxanthine-guanine/ phosphoribosyltransferase deficit) and some cases of glycogen storage disease are associated with uricosuria.

2- Decreased in renal insufficiency, in some cases of glycogen storage disease (type I), and in any metabolic defect producing lacticacidemia or β-hydroxybutricacidemia. Salicylates in doses of less than 2-3 gm/day often produce renal retention of uric acid.

NORMAL CLINICAL VALUES: BLOOD*

INORGANIC SUBSTANCES		TOTAL FATTYACIDS	190-420 mg/dl
Ammonia	12-55 µmol/l	Triglycerides	40-150 mg/dl
Bicarbonate	22-26 meq/l	Phenylalanine	0-2 mg/dl
Calcium	8.5-10.5 mg/dl	Pyruvic acid	0-0.11 meq/L
Carbon dioxide	24-30 meq/l	Urea nitrogen (BUN)	8-25 mg/dl
Chloride	100-106, eq/l	Uric acid	3.0-7.0 mg/dl
Copper	100-200 µg/dl	Vitamin A	0.15-0.6 µg/ml
Iron	50-150 µg/dl	PROTEINS	
Lead	10 µg/dl or less	Total	6.0-8.4 g/dl
Magnesium	1.5-2.0 meq/l	Albumin	3.1-4.3 g/dl
Pco2	35-45 mmHG	Ceruloplasmin	23-43 mg/dl
	4.7-6,0 kPa	Globulin	2.6-4.1 g/dl
Ph	7.35-7.45	Insulin	0-29µU/ml
Phosphorus	3.0-4.5 mg/dl		
Po2	75-100 mmHG	ENZYMES	
	10.0-13.3 kPa	Aldolase	0-7 U/ml
Potassium	3.5-5.0 meq/l	Amylase	4-25 U/ml
Sodium	135-145 meq/l	Cholinesterase	0.5 pH U or more/h
		Creatine kinase (CK)	40-150 U/L
ORGANIC		Lactic dehydrogenase	110-210 U/L
MOLECULES		Lipase	2 U/ml or less
Acetoacetate	Negative	Nucleotidase	1-11 U/L
Ascorbic acid	0.4-15 mg/dl	Phosphatase (acid)	0.1-0.63 Sigma U/ml
Bilirubin		Phosphatase (alkaline)	13-39 U/L
Direct	0-0.4 mg/dl	Transaminase (SGOT)	9-40 U/ml
Indirect	0.6 mg/dl		
Carotenoids	0.8-4.0 µg/ml	PHYSICAL	
Creatinine	0.6-1.5 mg/dl	PROPERTIES	
Glucose	70-110 mg/dl	Blood pressure	120/80 mmHG
Lactic acid	0.5-2.2 meq/l	Blood volume	8.5-9.0% of body weight in Kg
Lipids		Iron binding capacity	250-410 µg/dl
Total	450-1000 mg/dl	Osmolality	280-296 mOsm/Kg H_2O
Cholesterol	120-220 mg/dl	Hematocrit	37-52%
Phospholipids	9-16 mg/dl as lipid p		

NORMAL CLINICAL VALUE: URINE*

Acetoacetate (acetone)	0	Lead	120 µg/d or less
Amylase	24-76 U/ml	Phosphorus (inorganic)	Varies; average 1 g/d
Calcium	0-30 mg/d	Porphobilinogen	0
Copper	0-60 µg/d	Protein (quantative)	Less than 165 mg/d
Coproprphyrin	50-250 µg/d	Sugar	0
Creatine	Under 0.75 mmol/d	Titratable acidity	20-40 meq/d
Creatinine	15-25 mg/kg body weight/d	Urobilinogen	Up to 1.0 ehrlich
5-Hydroxyindoleacetic acid	2-9 mg/d	Uroporphyrin	0-30 µg/d